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Responsiveness of Cerebral Osmoreceptors in the Anesthetized Dog (41504)

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Abstract. Experiments were carried out to determine if, in the anesthetized dog undergoing a water diuresis, selective elevation of cerebral osmolality induced an antidiuresis. Bilateral intracarotid infusion of hypertonic sodium chloride in an amount previously shown to increase jugular plasma osmolality by 3% was associated with a significant decline in C_{H_2O}. The same volume of hypertonic sodium chloride infused intravenously did not alter C_{H_2O} significantly. These experiments do not support the view that anesthesia blocks the response of cerebral osmoreceptors.

The concept that cerebral osmoreceptors modulated the secretion of antidiuretic hormone (ADH) was suggested by Verney as the result of studies done in the 1940s (1). He observed that intracarotid bolus injections of hypertonic NaCl inhibited a water diuresis in the conscious dog. Since the antidiuretic response could be mimicked by the injection of posterior pituitary extract, Verney suggested that elevation of cerebral osmolality caused shrinkage of osmoreceptor cells which, in turn, led to the release of ADH.

These studies were not seriously challenged until the work of Bie. He found that in anesthetized dogs, elevation of cerebral osmolality by an estimated 5% had no effect on a water diuresis (2). In addition, the time course and extent of the antidiuresis produced by an ic or iv 60-min infusion of a hypertonic NaCl solution was the same. Although at this time Bie did not deny the possibility that Verney was correct, he questioned if the response observed by Verney was specific.

Bie has provided an in-depth exhaustive review of the concept of osmoreceptors, in which he reaffirms his position that the stimuli used in osmoreception studies have not been shown to be physiological osmostimuli (3). However, a very important part of the review is provided as an addendum in which a study by Dietz *et al.* (4) is discussed. This group observed that in the conscious dog, a bilateral ic, 10-min infusion of hypertonic NaCl that was shown to increase jugular osmolality by only 3%, inhibited a water diuresis. Since Dietz *et al.* used conscious dogs and Bie anesthetized dogs, Bie suggested that his failure to obtain results consistent with a central osmoreceptor hypothesis was due to the fact that he used anesthetized dogs while Verney and Dietz *et al.* used conscious dogs, i.e., anesthesia blocks the cerebral osmoreceptor mechanism. To test this hypothesis, we carried out experiments in a group of anesthetized dogs, including those used by Dietz *et al.*, to determine if, when anesthetized with the same anesthesia used by Bie, they would show an antidiuresis when cerebral osmolality is elevated within a physiologic range. Contrary to Bie's hypothesis, the results show that anesthesia does not block the cerebral osmoreceptor mechanism.

Methods. The study was conducted using a group of female American Foxhounds weighing between 20 and 27 kg which had been prepared with bilateral carotid loops. The dogs were fasted for 18 hr before an experiment.

They were anesthetized with 60 mg/kg α-chloralose (Sigma Chemical) and 10

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mg/kg sodium pentobarbital (Nembutal, Abbott) administered via an intracath placed in a saphenous vein and advanced to the level of the right heart. A Foley catheter was placed in the bladder *via* the urethra and the carotids were catheterized with 2-in., 18-gauge angiocaths. Blood pressure and heart rate were monitored via one carotid line except during the bilateral IC infusion.

A steady state water diuresis was established by infusing a volume of hypotonic solution of 25 m*M* urea and 40 m*M* glucose equivalent to 2% of body weight over a period of one hour. To maintain the diuresis, the urine output in any given period was matched by infusing an equal amount of glucose–urea solution.

When a steady state urine flow rate was achieved, a 10-min, bilateral ic or a 10-min iv infusion of hypertonic NaCl was performed. The NaCl solution was infused at a rate of 45 μM/kg body weight/min/artery or 90 μM/kg/min intravenously. Blood samples for electrolytes, osmolality, creatinine concentration, and, in some experiments, arginine vasopressin (ADH) radioimmunoassay (6) were taken immediately before and 9 min into the infusion period, and again at 60 min after the hypertonic infusion. At the end of the experiments, the animals were returned to their kennel to recover from the anesthesia.

Data were analyzed using one-way analysis of variance for repeated measures. Significance reported is $P < 0.05$.

Results. In five dogs given 90 μM/min/kg NaCl intravenously (Fig. 1B), no significant change occurred from the mean control creatinine clearance (GFR) of 52 ml/min. Free water clearance (C_{H_2O}) (control mean = 2.2 ml/min), urinary sodium excretion ($UNa\dot{V}$) (control = 13.7 μeq/min), and urinary potassium excretion ($UK\dot{V}$) (control = 8.1 μeq/min) did not change significantly from control values. Heart rate (HR) and blood pressure (BP) did not change. Plasma sodium concentration increased from a control of 141 meq/liter to 143 meq/liter after the hypertonic NaCl infusion and remained elevated for the duration of the experiment. Plasma osmolality increased from 290 to 294 mOsm/liter. In the three experiments in which it was measured,

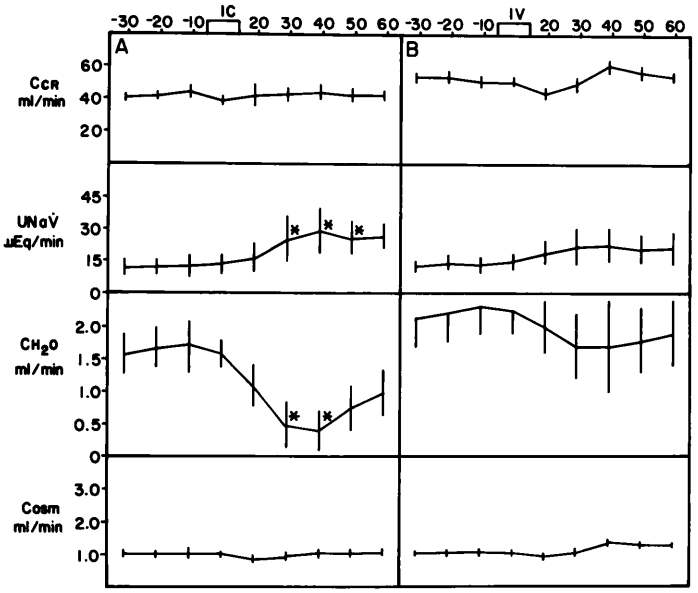

FIG. 1. Results of ic (Panel A) and iv (Panel B) infusions of hypertonic NaCl solution. C_{cr}, creatinine clearance; $UNa\dot{V}$, urinary sodium excretion; C_{H_2O}, free water clearance; C_{osm}, urinary osmolar clearance. Asterisks indicate statistical significance, $P < 0.05$.

ADH concentration remained less than 1.25 pg/ml.

The response to bilateral ic infusion of hypertonic NaCl in eight dogs is shown in Fig. 1A. Creatinine clearance remained near the control value of 42 ml/min throughout the experiments. UNa$\dot{V}$ increased significantly by 30 min after the beginning of the ic infusion, to a peak value of 28.8 μeq/min from a control level of 12.5 μeq/min. C_{H_2O} decreased significantly by 30 min after the beginning of the ic infusion from a control level of 1.7 ml/min to a minimum of 0.4 ml/min indicating a pronounced antidiuretic effect of the ic infusion. No significant change occurred from a control UK$\dot{V}$ of 11.3 μeq/min, or in HR or BP. Plasma sodium concentration showed precisely the same increase as with the iv infusion, that is, from 141 meq/liter to 143 meq/liter. Plasma osmolality increased from 288 to 292 mOsm/liter. In the six experiments in which it was measured, ADH concentration remained less than 1.25 pg/ml.

Discussion. The results clearly show that selective increases in cerebral osmolality in the chloralose anesthetized dog is associated with a significant antidiuresis. These results are consistent with the results of Verney (1) and Dietz *et al.* (4) in the conscious dog but inconsistent with the results of Bie (2, 3) in the anesthetized dog. Bie infused 83 μM/kg/min ic, bilaterally for 8 min and observed an increase in free water despite the fact that by the end of the infusion, peripheral venous osmolality had increased by approximately 7 mOsm/kg. The increase (4 mOsm/liter) in peripheral osmolality in the present experiments caused by an ic infusion was associated with an antidiuresis. However, the peripheral plasma osmolality in Bie's experiments was 283.6 mOsm/liter while in the present experiments, it was 290 (iv) and 288 (ic) prior to the hypertonic sodium chloride infusion.

Using dehydration as a stimulus in conscious dogs, Robertson (5) found that the threshold P_{osm} for ADH release was 286 mOsm/liter while we found under these conditions a value of 297 mOsm/liter (Huffman and Gilmore, unpublished observations). Studies in man and rats indicate that under conditions of hypervolemia (as in the present experiments and those of Bie due to the induction of a H_2O diuresis), the osmotic threshold for ADH release would be increased. It is, therefore, quite possible that in the Bie experiments, because of the low resting P_{osm} (283.6), the hypertonic NaCl infusions did not cause P_{osm} to reach the osmotic threshold for ADH release while in the present experiments, because of the higher P_{osm}, threshold was reached and thus an antidiuresis ensued. The reason why the resting P_{osm} in Bie's experiments in which he infused hypertonic NaCl for 8 min was lower than in the present experiments is probably because he produced a 4% body weight hydration while we produced a 2% body weight hydration. Again, this greater state of hydration (hypervolemia) would be expected to produce a greater increase in the osmotic threshold for ADH release. The diuresis observed by Bie might be explained as the result of increased fluid delivery to distal nephron sites resulting in an increased generation of C_{H_2O}.

A major obstacle to conclusive results in studies such as these is the difficulty in measuring changes in ADH. Because of the hydration, even the relatively moderate 2% of body weight used in the present study, ADH is suppressed below concentrations at which changes from steady state, control concentrations can be measured accurately. From the ADH data reported under Results, it can be concluded that the 2% hydration was sufficient to suppress ADH release in response to the hydration. Whether any increase in ADH occurred due to ic or iv hypertonic NaCl infusion cannot be determined; it can only be stated that any increase was not sufficient to raise plasma ADH above 1.25 pg/ml, the lower limit of the assay used (6).

In summary, the present study demonstrates the responsiveness of cerebral osmoreceptors during chloralose/pentobarbital anesthesia, supporting the cerebral osmoreceptor concept of Verney (1).

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